REMARKS

Claims 1-7, 10, 14, and 15 are pending in the application.

Applicants have replaced each occurrence of "may be" in claims 1 and 15 with either "is" or "are." Applicants have deleted "or a pharmaceutically acceptable salt thereof" in claim 15. No new matter is introduced by these amendments.

Rejection under 35 U.S.C. § 103

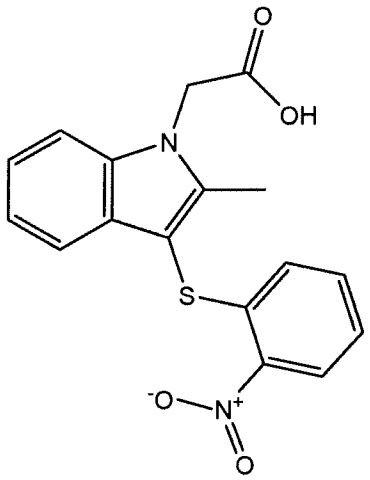
Claim 15 is rejected under 35 U.S.C. § 103 as being unpatentable over "*Luescher, et al.* (See RN 86704-63-04, CAPLUS)" (Office Action, page 3).

[1] It is Applicants' understanding, based on the abstract attached to the Office Action, that "*Luescher, et al.*" refers to the following journal article:

Lüscher, I.F. and Schneider, C.H. "**51. Deblocking of *o*-Nitrophenylsulfenyl-Protected Peptides by Ammonium Thiocyanate and (2-Methyl-1-indolyl)acetic acid**" *Helv. Chim. Acta.* **1983**, *66*, 602-605 ("Lüscher").

A copy of the above-mentioned journal article is included with this Reply. For purposes of clarification, reference to "Lüscher" in the discussion below refers to the above-mentioned journal article and not the abstract attached to the Office Action.

[2] The Office appears to rely on the disclosure of "(2-methyl-3-(2-nitrophenylthio)-1-indolyl) acetic acid" in Lüscher as the basis for the rejection (see pages 3-5 of the Office Action). For ease of exposition, the aforementioned compound is referred to in the discussion below as "the Lüscher compound." The chemical structure of the Lüscher compound is shown below:



According to the Office (Office Action, pages 4-5, emphasis added):

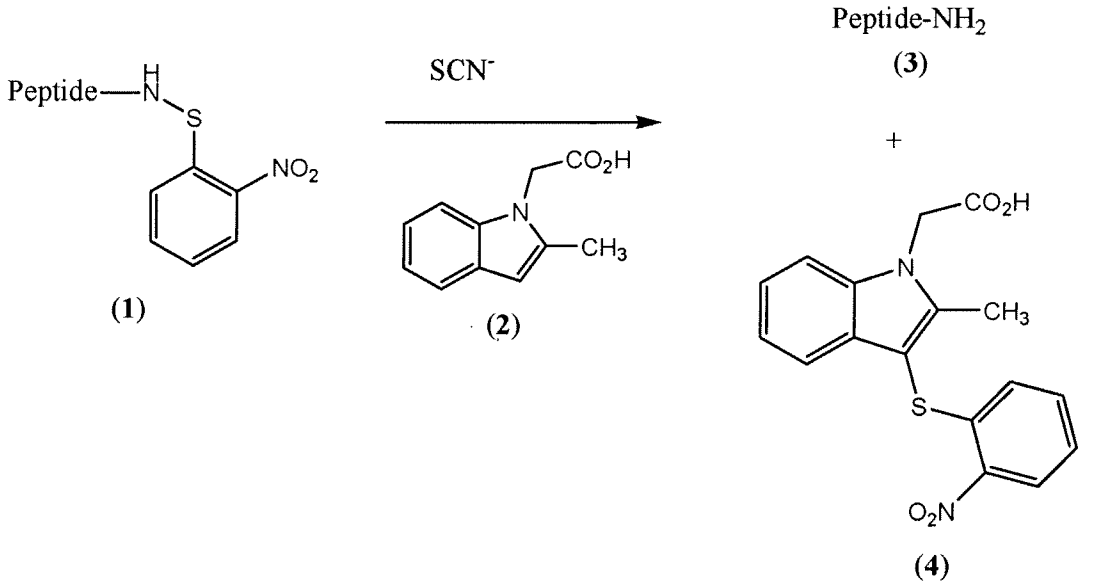
> [I]t is obvious to prepare an indole derivative wherein the benzene ring is unsubstituted by an alkyl (i.e. methyl) when the art teaches a similar compound wherein the benzene ring is unsubstituted with a reasonable expectation of success. Specifically, adding a methyl substituent to the benzene ring with the same core structure as taught in the prior art is obvious absent unexpected results. Therefore, it would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to prepare adjacent homologs based on the teachings of the **preferred** embodiments in the prior art. A strong prima facie obviousness has been established.

This is respectfully traversed.

[3] Lüscher

Lüscher is concerned exclusively with procedures for deblocking *o*-nitrophenylsulfenyl (referred to in the discussion below by the acronym "Nps")-protected peptides. In particular, Lüscher discloses a deblocking procedure that includes exposing an Nps-protected peptide (compound (**1**) in Scheme 1 below) to thiocyanate ion (i.e., SCN^-) and (2-methyl-1-indolyl) acetic acid (compound (**2**) in Scheme 1 below) or a salt of compound (**2**).

Scheme 1



(**1**) (**2**) (**4**)

(i.e., the Luscher compound)

In addition to providing the desired deprotected peptide (**3**), the Lüscher deblocking procedure described above also generates a by-product (**4**). As indicated in Scheme 1, this reaction by-product (**4**) is **the Lüscher compound**. Thus, the Lüscher compound, which is the **same** compound relied upon by the Office in the present rejection, is a by-product that is formed in this peptide deprotection reaction. Thus, the Lüscher compound is effectively a contaminant that must be removed in order to obtain the desired deprotected peptide in purified form.

Lüscher does not disclose any apparent utility for the Lüscher compound. Apart from the brief experimental section of Lüscher, the only other mention of the Lüscher compound in the reference concerns the facility with which the Lüscher compound can be extracted into "organic solvents" and "aqueous base" (see Lüscher at page 603 at the paragraph immediately following Table 1 and its accompanying footnotes). Finally, there is no indication that Lüscher does anything at all (much less anything useful) with the Lüscher compound once it is removed from the crude deblocking product mixture *via* extraction (see Lüscher at e.g., page 602 in the "*Summary*" and the experimental section on pages 604 and 605).

[4] The Federal Circuit in *Eisai Co. Ltd. v. Dr. Reddy's Laboratories, Ltd.* 533 F.3d 1353, 1358 (2008) discussed the requirements for establishing whether a claimed compound is *prima facie* obvious over a reference compound (emphasis added):

> The Supreme Court's analysis in *KSR* thus relies on several assumptions about the prior art landscape. First, *KSR* assumes a starting reference point or points in the art, prior to the time of invention, from which a skilled artisan might identify a problem and pursue potential solutions. Second, *KSR* presupposes that the record up to the time of invention would give some reasons, available within the knowledge of one of skill in the art, to make particular modifications to achieve the claimed compound. *See Takeda*, 492 F.3d at 1357 ("**Thus, in cases involving new chemical compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound**."). Third, the Supreme Court's analysis in KSR presumes that the record before the time of invention would supply some reasons for narrowing the prior art universe to a "finite number of identified, predictable solutions," 127 S.Ct. at 1742. In *Ortho-McNeil Pharmaceutical, Inc. v. Mylan Laboratories, Inc.*, 520 F.3d 1358, 1364 (Fed.Cir.2008), this court further explained that this "easily traversed, small and finite number of alternatives ... might support an inference of obviousness." To the extent an art is unpredictable, as the chemical arts often are, *KSR's* focus on these "identified, predictable solutions" may present a difficult hurdle because potential solutions are less likely to be genuinely predictable.
>
> **In other words, post- *KSR*, a prima facie case of obviousness for a chemical compound still, in general, begins with the reasoned identification of a lead compound**.

[5] Applicants submit that the teachings of Lüscher would not have led one to select the Lüscher compound (or any other compound in Lüscher) as a lead compound for any purpose. This is discussed in more detail below.

The Lüscher compound is nothing more than a by-product of a peptide deprotection reaction. Thus, it is effectively a contaminant that must be removed in order to obtain the desired deprotected peptide in purified form. Lüscher does not disclose any apparent utility for the Lüscher compound. Apart from the brief experimental section of Lüscher, the only other mention of the Lüscher compound in the reference concerns the facility with which the Lüscher compound can be extracted into "organic solvents" and "aqueous base" (see Lüscher at page 603 at the paragraph immediately following Table 1 and its accompanying footnotes). Finally, there is no indication that Lüscher does anything at all (much less anything useful) with the Lüscher compound once it is removed from the crude deblocking product mixture *via* extraction (see Lüscher at e.g., page 602 in the "*Summary*" and the experimental section on pages 604 and 605).

In view of the above, the Lüscher compound does not at all appear to fall within the purview of "preferred embodiments" as is asserted by the Office. As such, it would not have been obvious to select the Lüscher compound (or any other compound in Lüscher) as a lead compound for further study or use. Accordingly, it also would not have been obvious to a person of ordinary skill in the art to make any kind of variant of the Lüscher compound. Thus, one of ordinary skill in the art would not be motivated by Lüscher to prepare the compounds claimed in claim 15 for at least this reason.

Applicants therefore respectfully request that the rejection be reconsidered and withdrawn.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 1-7, 10, 14, and 15 are rejected under 35 U.S.C. § 112, second paragraph for allegedly being indefinite. The recitation of "may be optionally substituted" appears to the basis for the rejection.

Applicants respectfully disagree with the grounds for the rejection; however, to expedite prosecution, Applicants have replaced each occurrence of "may be" in claims 1 and 15 with either "is" or "are."

Applicants therefore respectfully request that the rejection be reconsidered and withdrawn.

CONCLUDING FORMALITIES

Applicants submit that all claims are in condition for allowance.

The fee in the amount of $130 for the one month extension fee is being paid concurrently herewith on the Electronic Filing System (EFS) by way of a Deposit Account authorization. Please apply any other charges or credits to deposit account 06-1050, referencing Attorney Docket No. 06275-435US1 / 100770-1P US.

Respectfully submitted,

Date: March 13, 2009

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51. Deblocking of *o*-Nitrophenylsulfenyl-Protected Peptides by Ammonium Thiocyanate and (2-Methyl-1-indolyl)acetic acid

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(6.XII.82)

Summary

The thiocyanate cleavage of the N^{α}-*o*-nitrophenylsulfenyl group from peptides in solution or on a solid support proceeds effectively in the presence of (2-methyl-1-indolyl)acetic acid. This scavenger was prepared from 2-methylindole and sodium bromoacetate; it can readily be removed by extraction with base after the cleavage reaction, together with (2-methyl-3-(2-nitrophenylthio)-1-indolyl)acetic acid.

The *o*-nitrophenylsulfenyl (Nps) group introduced into peptide synthesis by *Zervas et al.* [1] has considerable potentialities as a temporary protecting group for the α-amino terminus because its selective removal can be accomplished by nucleophilic reagents. These reagents avoid problems encountered with protecting groups requiring acids for their cleavage, *i.e.* alkylation of the side chains of methionine, tyrosine and also lysine and histidine by carbocations or their reaction products with the deprotecting reagent (*e.g. t*-butyl trifluoroacetate) [2]. Thiolytic cleavage of the Nps group with a number of reagents has been described, and recently 2-mercaptopyridine was shown to enable rapid deprotection [3] [4].

In our hands the method of *Wünsch & Spangenberg* [5] using thiocyanate (rhodanide) in the presence of 2-methylindole proved very valuable for the cleavage of Nps-protected peptides. Thiocyanate ion attacks *N*-terminally bound Nps and reversibly forms *o*-nitrophenylsulfenyl thiocyanate (**1**). The equilibrium is fully displaced in the presence of excess 2-methylindole since **1** is converted into stable 3-(2-nitrophenylthio)-2-methylindole (**2**) and thiocyanate ion. The indole derivative is removed by washing with ether. However, with relatively lipophilic peptides, ether extraction becomes unsatisfactory. Since the two-phase-purification method of peptide synthesis [6] [7] frequently used in our laboratory depends on lipophilic peptide intermediates, it seemed worthwhile to adapt the indole reagent used in [5] and to investigate carboxy derivatives of indole which can be removed by aqueous base after reaction with an Nps residue.

Using Nps-Lys(Boc)-OH as a model, the cleavage rate of a variety of reagents was assessed *(Table 1)*. It is obvious that the 3-position of the indole system should be reserved for the Nps capture and cannot be blocked as in the indoles **3**–**5**. The

substitution at the N-atom of 2-methylindole with a carboxymethyl or carboxylatomethyl group is, on the other hand, advantageous, and compounds **7** and **7a** are as reactive as 2-methylindole.

Table 1. *Removal of the Nps group from Nps-Lys(Boc)-OH dicyclohexylammonium salt with NH_4SCN in the presence of different indole derivatives*

Nr.	Indole derivative	Time for complete reaction[a])
3	(3-Indolyl)acetic acid	> 48 hours
4	3-(3-Indolyl)propionic acid	40 hours
5	L-Tryptophane	> 48 hours
6	2-Methylindole	3 min
7	(2-Methyl-1-indolyl)acetic acid	3 min
7a	Dicyclohexylammonium (2-methyl-1-indolyl)acetate	3 min

[a]) Nps-Lys(Boc)-OH dicyclohexylammonium salt (0.1 mmol) in 3.8 ml of $CH_2Cl_2/CH_3OH/CH_3COOH$ 15:2:2 was mixed with 0.2 mmol of NH_4SCN and 0.2 mmol of indole derivative and stirred at r.t. (approx. 23°). The final volume was 3.9 ml and the molarities therefore 0.0255 and 0.051 for the lysine derivative and the reagents, respectively. Frequently 2 µl aliquots of the reaction solution were withdrawn and immediately chromatographed on silica gel plates (*F254, Merck*, Darmstadt) with $CHCl_3/CH_3OH$ 9:1. The time of disappearance of Nps-Lys(Boc)-OH (Rf 0.4) detected by fluorescence quenching at 254 nm is taken as the time for complete reaction.

The synthesis of **7** and **7a** from the sodium salt of 2-methylindole proceeded essentially according to *Cardillo et al.* [8], who reported alkylations of the indole sodium salt. The reaction with sodium bromoacetate in heterogeneous phase is not adapted for maximum yield. Mostly the salt **7a** was employed for deprotection. The Nps-substituted product (2-methyl-3-(2-nitrophenylthio)-1-indolyl)acetic acid (**8**) was also prepared and shown to be a stable compound of good solubility in many organic solvents and easily extractable from such solutions with aqueous base.

Table 2. *Removal of the* N*-terminal Nps group from various peptides with NH_4SCN in the presence of dicyclohexylammonium 2-methylindol-1-yl acetate* (**7a**)

Peptide[a])	Time for complete cleavage[b]) [min]
Nps-Lys(Boc)-OSuco	4
Nps-Lys(Boc)-Lys(Boc)-OSuco	7
Nps-Lys(Boc)-[Lys(Boc)]$_3$-Lys(Boc)-O*t*Bu	50
Nps-Lys(Boc)-[Lys(Boc)]$_4$-Lys(Boc)-OSuco	40
Nps-Lys(Boc)-[Lys(Boc)]$_6$-Lys(Boc)-OSuco	60
Nps-Lys(Boc)-ε-Ahx-[Lys(Boc)-ε-Ahx]$_3$-Lys(Fmoc)-Gly-OSuco	60
Nps-Leu-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt	30

[a]) OSuco: 3-[4-(5α-cholestan-3β-yl)]$OCH_2C_6H_4CH_2-O-CO-CH_2CH_2COO$; ε-Ahx: 6-aminohexanoic acid; Fmoc = 9-(Methoxycarbonyl)fluorenyl.

[b]) The method of *Table 1* was used. Where appropriate, TLC. was performed with toluene/EtOH 7:3 instead of $CHCl_3/CH_3OH$.

As shown in *Table 2*, the thiocyanate cleavage in the presence of **7a** becomes slower when peptides of increasing size are treated. However, in all cases virtually

homogeneous N^a-deprotected peptides were obtained after extraction of the reaction solution with H_2O, 0.2 M K_2CO_3 and 0.1 M HCl. Interestingly, Nps-Lys(Boc) bound to a standard solid support could be N^a-deprotected within a relatively short time by treating the resin first with NH_4SCN alone, adding **7a** after several minutes.

The authors thank Dr. *P. Bigler* for ^{1}H-NMR. analyses. This work was supported by the *Swiss National Science Foundation.*

Experimental Part

General. Amino acid derivatives, reagents, solvents, (3-indolyl)acetic acid, 3-(3-indolyl)propionic acid and 2-methylindole were obtained from *Fluka,* Buchs. NaH was washed with hexane before suspension in dry THF. THF was passed through an aluminium oxide *(504C, Fluka)* column before use. Peptides were prepared according to the two-phase-purification method [6] [7] and were taken from ongoing projects. Nps-Lys(Boc)-O-resin was prepared from polystyrene resin, *Merrifield* type, 1% DVB, containing 1.2 mol-equiv. of chloromethyl groups per g, and Nps-Lys(Boc)-O-Cs^+ using the method of *Juillerat & Bargetzi* [9] for preparing Nps-Gly-O-resin. Nps-Cl liberation with HCl indicated the binding of 0.38 mol-equiv. per g. Thin layer chromatography (TLC.) was performed on fluorescent 5 × 10 cm silica gel plates *60F254, Merck,* Darmstadt, with $CHCl_3/CH_3OH$ 9:1 (A) or 92.5:7.5 (B) or toluene/EtOH, 7:3 (C). Spots were detected as described previously [10]. Melting points are uncorrected. UV. spectra were recorded on a *Pye Unicam SP 8-100* spectrophotometer. ^{1}H-NMR. spectra were obtained in CD_3COCD_3 from a *Varian XL 100* spectrometer using tetramethylsilane as internal standard. Elementary analysis were performed by *H. Frohofer,* University of Zürich.

Preparation of dicyclohexylammonium (2-methyl-1-indolyl)acetate (**7a**). A solution of 2-methylindole (22.5 g, 0.172 mol), recrystallized from EtOH/H_2O, dissolved in 70 ml of THF was added dropwise with exclusion of moisture under N_2 to NaH (8.24 g, 0.343 mol) suspended in 80 ml of THF. The suspension was refluxed for 30 min, and after cooling, $BrCH_2COONa$ (27.6 g, 0.17 mol) suspended in 70 ml of THF was added within 15 min under vigorous stirring. Then refluxing and stirring under N_2 was continued for 2 h. To the cold (basic) solution 300 ml of H_2O was added dropwise, the mixture extracted 10 times with a total of 500 ml of $CHCl_3$, acidified with 6 M HCl to pH 2 and finally extracted with 600 ml of EtOAc in 6 portions. The org. phase was washed 4 times with 50 ml portions of 0.1 M HCl and H_2O and dried with Na_2SO_4. Dicyclohexylamine was then added to pH 7, and after standing overnight at 4°, the colorless, virtually scentless needles were filtered off, washed with EtOAc and dried *in vacuo:* 38.5 g (61%) of **7a**, m.p. 214–217°.

$C_{23}H_{34}N_2O_2$ (370.5) Calc. C 74.60 H 9.24 N 7.60% Found C 74.86 H 8.95 N 7.64%

Preparation of (2-methyl-1-indolyl)acetic acid (**7**). The free acid was obtained by extracting a solution of **7a** in CH_2Cl_2 with $KHSO_4$-solution according to [11]. Removal of CH_2Cl_2 *in vacuo* left **7** as a crystalline material, m.p. 206–208°. TLC. (A): Rf 0.32, homogeneous; (C): Rf 0.44, homogeneous. – UV. (EtOH): 220 (27700), 274 (7200), 280 (7300), 289 (5800). – ^{1}H-NMR.: 7.51–7.21 (*m*, 2 H, H–C(5), H–C(8)); 7.20–6.91 (*m*, 2 H, H–C(6), H–C(7)); 6.25 (*d* × *qa*, 1 H, H–C(3)); 5.88 (br. *s*, COOH, H_2O); 4.94 (*s*, 2 H, $CH_2N(1)$); 2.39 (*s*, 3 H, H_3C–C(2)).

Preparation of (2-methyl-3-(2-nitrophenylthio)-1-indolyl)acetic acid (**8**). Nps-Gly-OH dicyclohexylammonium salt (410 mg, 1.0 mmol) dissolved in 30 ml of CH_2Cl_2, 4 ml of CH_3OH and 4 ml of CH_3COOH was stirred under Ar in the dark with 152 mg (2.0 mmol) of NH_4SCN and 180 mg (0.95 mmol) of **7** for 1 h. The solution was diluted with 300 ml of CH_2Cl_2 and extracted in a spray column extractor [6] with 1 l of 0.1 M HCl, 0.3 l of H_2O and 1.5 l of 0.2 M K_2CO_3. The K_2CO_3-extract was mixed with 100 ml of EtOAc and acidified under efficient stirring with hydrochloric acid to pH 2. The AcOEt layer was dried with Na_2SO_4 and evaporated *in vacuo:* 458 mg (92%) of **8** as orange powder, m.p. 202–204°. TLC. (A): Rf 0.18, homogeneous; (C): Rf 0.29, homogeneous. – UV. (EtOH): 222 (35000), 280 (12200), 289 (10200), 371 (3500). – ^{1}H-NMR.: 8.34–8.24 (*m*, 2 H, H–C(3'), H–C(5')); 7.64–7.03 (*m*, 4 H, H–C(5), H–C(6), H–C(7), H–C(8)); 6.96–6.84 (*m*, 2 H, H–C(4'), H–C(6')); 5.22 (*s*, 2 H, $CH_2N(1)$); 2.53 (*s*, 3 H, H_3C–C(2)).

Preparative removal of Nps from Nps-Lys(Boc)-[Lys(Boc)]$_6$-Lys(Boc)-OSuco. The protected peptide (5.56 g, 2.15 mmol) dissolved in 80 ml of $CH_2Cl_2/CH_3OH/CH_3COOH$ 15:2:2 was stirred with 4.30 mmol of NH_4SCN and 4.30 mmol of **7a** at r.t. in the dark for 70 min. After dilution with 300 ml of CH_2Cl_2 the solution was extracted in a spray column extractor [6] with 1 l of 0.1M HCl, 0.3 l of H_2O, 2 l of 0.2M K_2CO_3 and 0.5 l of H_2O. Removal of the solvent and drying *in vacuo* gave a colorless residue: 5.18 g (99%) of H-Lys(Boc)-[Lys(Boc)]$_6$-Lys(Boc)-OSuco. TLC. (A): Rf 0.30, homogeneous; (B): Rf 0.18, homogeneous.

Removal of Nps from Nps-Lys(Boc)-O-resin. To Nps-Lys(Boc)-O-resin (50 mg) stirred at r.t. in 19 ml of $CH_2Cl_2/CH_3OH/CH_3COOH$ 15:2:2, 0.50 mmol of NH_4SCN was added, followed by 0.57 mmol of **7a** after 5 min. After a total of 18 min, the resin was filtered off and washed with solvent to give a colorless product.

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